Synthesis and Rheological Characterization of Disulfide Crosslinked Hyaluronan Hydrogels

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Statement of Purpose: Hyaluronan (HA, Fig. 1), a major component of the natural extracellular matrix, plays essential roles in cell migration, tissue development, angiogenesis, and wound healing [1-2]. Chemically modified HA and HA hydrogels have been investigated as scaffolds for tissue engineering of skin and cartilage [3]. Here we introduce a new strategy for synthesis of HA hydrogels crosslinked through polyethylene glycol (PEG) via disulfide exchange reaction (Fig. 1), aiming to develop mechanically tunable and bio-interactive materials for tissue engineering and regenerative medicine.

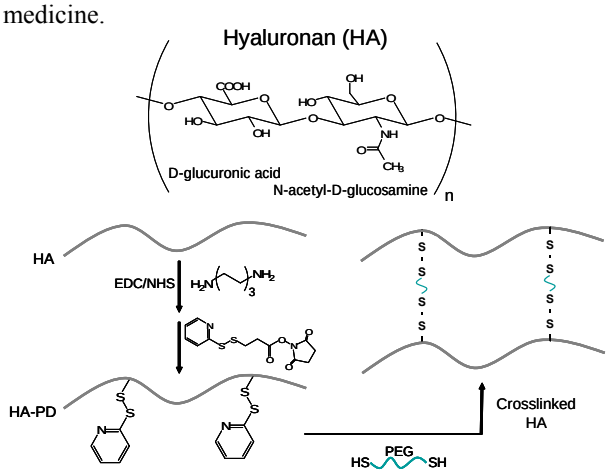

Fig. 1. Synthesis of HA hydrogel via disulfide exchange

Methods: HA (MW 1.1-1.4x10^6, Lifecore, Chaska, MN) was conjugated with 1,6-diaminohexane using carbodiimide chemistry [4], followed by reaction w ith N-succinimidyl 3-(2-pyridyldithio)-propionate (SPDP, Pierce, Rockford, IL) [5], yielding HA-pyridyl disulfide (HA-PD). Hydrogels were synthesized by crosslinking HA-PD with various amounts of PEG-dithiol (MW 3,400, Nektar, Huntsville, AL) in PBS (pH 7.4) at 37°C. Final concentration of HA in the hydrogels was 1.0% (w/v). Rheological testing was performed on an AR-G2 rheometer (TA Instruments, Inc., New Castle, DE) using a standard parallel-plate geometry. Oscillatory time, frequency, and strain sweeps were performed at 37°C. Normal human dermal fibroblasts (NHDFs) were suspended in media, mixed with HA-PD and PEG-dithiol, and allowed to gel at 37°C. After culturing for 5 days, the encapsulated cells were examined by live/dead staining with calcein Am and ethidium homodimer to assess cell viability.

Results/Discussion: A disulfide exchange reaction was used to crosslink HA with PEG. The gelation was rapid and potentially quantifiable. Oscillatory time sweep data was used to determine gelation point as ~5-7 minutes (Fig. 2), which is faster than HA gels prepared by thiol oxidation and comparable to gels crosslinked via Michael-type addition [6], while still yielding reversible disulfide bonds. Frequency and strain sweeps showed that elastic modulus and ultimate stress level of hydrogels increased with increasing theoretical crosslink density (Fig. 3). Primary human dermal fibroblasts were successfully encapsulated in situ in the HA/PEG gels. Live/dead staining indicated that most cells were viable after encapsulation and culture within the hydrogels.

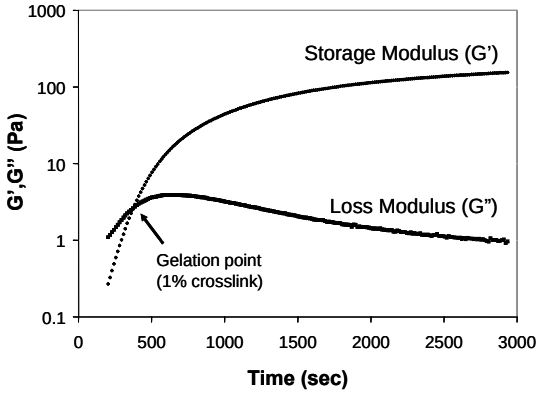

Fig. 2. Rheological determination of gelation point

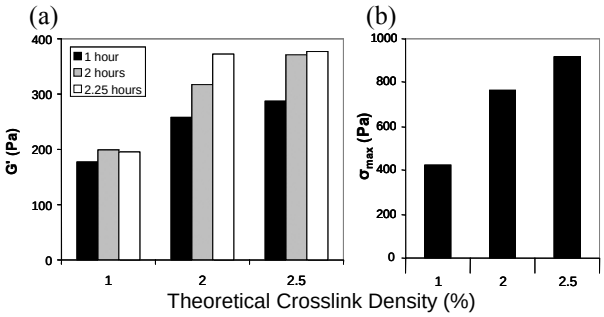

Fig. 3. Dependence of elastic modulus G' (a) and ultimate stress level σ_{max} (b) on gelation time and crosslink density

Conclusions: We have introduced a new strategy for convenient synthesis of in situ crosslinked HA hydrogels with tunable rheological properties, and demonstrated that rapid gelation is appropriate for cell encapsulation. This hydrogel system potentially allows for incorporation of cell-interactive signals through disulfide exchange reaction, and represents a versatile material platform for tissue engineering and regenerative medicine.

References:

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